Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that causes debilitating central neuropathic pain (CNP) in many patients. Although mouse models of experimental autoimmune encephalomyelitis (EAE) have provided insight on the pathobiology of MS-induced neuropathic pain, concurrent severe motor impairments confound quantitative assessment of pain behaviours over the disease course. Hence, my PhD research project was designed to establish and characterise an optimised mouse model of multiple sclerosis (MS)-induced neuropathic pain using behavioural, pharmacological, histological and immunohistochemical methods. I then used my optimised EAE-mouse model of MS-neuropathic pain to gain insight on the pathobiology of MS-associated neuropathic pain. In the final part of my PhD research project, I investigated the analgesic efficacy and mode of action of 'Treatment-A' as a novel treatment for relief of MS-neuropathic pain.

EAE-rodent models induced by immunisation with myelin oligodendrocyte glycoprotein (MOG) are commonly used for research on MS due to their CNS pathological features in common with patients with MS. However, a major limitation of most of previously published MOG-induced EAE-mouse models is severe motor impairment (e.g. hindlimb paralysis) that confounds assessment of behavioural pain endpoints as the disease progresses. To address this issue, I systematically optimised the various components of the immunisation protocol for induction of mild relapsing-remitting EAE disease in C57BL/6 female mice. My optimised immunisation protocol comprised MOG_{35-55} (200 µg) together with adjuvants including Quil A (45 µg) and pertussis toxin (2 x 250 ng). Quil A replaced the traditionally used Freund's Complete adjuvant (FCA), as FCA itself may induces CNS neuroinflammation. Apart from inducing a mild relapsing-remitting EAE (RR-EAE) clinical disease course in mice, my optimised immunisation protocol also induced temporal development of mechanical allodynia in the bilateral hindpaws, but without confounding motor deficits. Mechanical allodynia was fully developed in the hindpaws of my RR-EAE mice by 28-35 days post-immunisation (p.i.) and it was maintained until study completion (55-57 days p.i.).

Next, I pharmacologically characterised my optimised EAE-mouse model of MS-neuropathic pain using clinically available analgesic agents. Single bolus doses of amitriptyline, gabapentin and morphine (but not vehicle) produced dose-dependent relief of mechanical

allodynia in the bilateral hindpaws of EAE-mice; the corresponding ED_{50}s were 1.5, 20 and 1 mg/kg respectively.

I then used histological and immunohistochemical methods to further characterise my optimised EAE-mouse model of MS-neuropathic pain. For this purpose, brain and lumbar spinal cord tissues were collected from EAE-, sham- and age-matched (non-immunised)-mice at day-39 p.i., a time when mechanical allodynia was fully developed in the bilateral hindpaws of EAE-mice. My findings showed significant demyelination, glial cell activation and augmented infiltration of $CD3^+$ T-cells into the CNS of RR-EAE mice (but not sham- or control-mice), mirroring these hallmark pathological features of MS in humans. Together, these findings show that my optimised RR-EAE mouse model has considerable potential for investigation of the pathobiological mechanisms underpinning MS-associated neuropathic pain. Furthermore, my optimised model is suitable for efficacy profiling of novel analgesics for improved relief of MS-induced neuropathic pain.

Upregulation of brain-derived neurotrophic factor (BDNF) and tyrosine kinsase (TrkB) has been implicated by others in the pathobiology of peripheral neuropathic pain; however it is unknown hitherto in CNP associated with MS. To address this knowledge gap, I next investigated the analgesic efficacy of 'Treatment-A', and the possibility that it may modulate lumbar spinal cord expression levels of BDNF and its receptor TrkB in my optimised EAE-mouse model of MS-induced neuropathic pain. Briefly, RR-EAE mice were administered once-daily subcutaneous (s.c.) doses of 'Treatment-A' (3 or 10 mg/kg/day) or vehicle over a 21 day period (till day-35 p.i.) commencing on the day of clinical disease relapse. Importantly, my findings showed that 'Treatment-A' dose-dependently and progressively alleviated mechanical hypersensitivity in the bilateral hindpaws of my RR-EAE mouse model of MS-induced neuropathic pain, whereas vehicle administered similarly, was without effect.

Finally, I showed that the cellular and molecular mechanisms underpinning the pain-relieving effects of chronically administered 'Treatment-A' at 10 mg/kg/day in RR-EAE mice involves a marked reduction in $CD3^+$T-cell infiltration and microglial activation in lumbar spinal cord to reduce augmented levels of BDNF-TrkB signalling and pp44/pp42 MAPK (also called pERK) that matched the respective levels in the lumbar spinal cord of vehicle-treated sham-mice. Importantly, the upregulated BDNF-TrkB-ERK signalling in RR-EAE mice treated with vehicle was confined to lamina-1 of the dorsal horn in the lumbar spinal cord.

In summary, during the course of my PhD, I have successfully established, optimised and characterised an RR-EAE mouse model of MS-induced neuropathic pain that is devoid of motor deficits. My findings also show that once-daily administration of ‘Treatment-A’ for 21 days according to an intervention protocol, progressively alleviated mechanical hypersensitivity in the bilateral hindpaws of RR-EAE mice in a dose-related manner. Treatment-A is worthy of further investigation as a potential novel treatment for relief of MS-induced neuropathic pain in patients.